DIETARY *N*-6 AND *N*-3 FATTY ACIDS ALTER THE FATTY ACID COMPOSITION OF TISSUES AND THE FATE OF BENEFICIAL FATTY ACIDS DURING PROCESSING

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***Abstract*—**The present study investigated the effects of dietary linolenic acid (ALA) *vs.* linoleic acid (LA) on fatty acid composition and stearoyl-CoA desaturase (SCD) activity in *longissimus* muscle and subcutaneous adipose tissue of bulls and on the fatty acid profile of German corned beef (GCB) and Tea sausage spread (TSS). Supplementation of the diet with ALA was accompanied by an increased level of *n*-3 fatty acids in muscle and fatty tissue which resulted in a decrease of *n*-6/*n*-3 ratio. The production of GCB by using lean meat of these bulls reflects the fatty acid transfer from beef to the product. The amount of ALA and the sum of *n*-3 LC PUFA in GCB are 1.4 times higher in the ALA enriched feeding group. This also caused a lower ratio of *n*-6/*n*-3 FA (4.0 *vs.* 5.9) in this group. The processing procedure did not affect negatively the fatty acid composition.

***Index Terms*—PUFA, SCD activity, German corned beef sausages, Tea sausage spread**

I. INTRODUCTION

It is necessary to supply linoleic (LA) and α-linolenic acid (ALA) by the diet because of the disability to synthesize these fatty acids *de novo* (Simopoulos 2008). Generally, *n*-6 and *n*-3 fatty acids are precursors for long-chain polyunsaturated fatty acids (LC PUFA) and for eicosanoids. This group of biologically active lipid mediators are known to mediate the inflammatory response (Palmquist 2009). Especially *n*-3 fatty acids have shown to have positive effects in breast cancer (Bougnoux, Hajjaji, Maheo, Couet & Chevalier 2010) and brain development (Coulson & Vitetta 2009). Conclusively, an adequate nutrient input and especially *n*-3 fatty acids in meat and meat products are recommended. Consumers are more and more interested in high quality meat products. Therefore, demands for higher quality increased the production of meat products with lower nitrate and fat content or enhanced LC PUFA (Verbeke, Pérez-Cueto, De Barcellos, Krystallis & Grunert 2010).

PUFA are also known to influence the lipogenic enzyme activity (Nakamura & Nara 2004). An important enzyme is the stearoyl-CoA desaturase (SCD). The SCD introduces a double bound in saturated fatty acids (SFA) to produce a monounsaturated fatty acid (MUFA) (Perfield *et al.* 2007) and is important to reduce SFA which have an increased risk of coronary heart disease (Rioux & Legrand 2007).

The aim of this study was to investigate the effect of LA and ALA feeding on fatty acid composition and the SCD activity in different tissues and to consider the transfer and the effect of processing on beneficial fatty acids from the fresh muscle to the beef product.

II. MATERIALS AND METHODS

Animal design

29 German Holstein bulls were assigned to two dietary treatments. The control group with 15 animals was fed with concentrate based on soybean meal and maize silage. This diet is high in LA. The experimental group received a diet high in ALA and this was achieved with a concentrate supplemented with rapeseed cake and linseed oil and grass silage. When compared both diets in terms of the quantity of LA and ALA the amount of LA is 1.4 times higher in control and ALA 4.0 times higher in experimental group. More details of the rations are described in Herdmann, Nuernberg, Martin, Nuernberg and Doran (2010).

Fatty acid analysis and analysis of CLA isomers by Ag^+ HPLC

Fat of *longissimus* muscle (2 g), subcutaneous fat (1 g) and sausages (2 g) were extracted with chloroform/methanol (2:1, v/v) by homogenization at room temperature and the fatty acid composition was determined with the methodology described by Nuernberg, Nuernberg and Dannenberger (2009). Four ChromSpher 5 Lipids silver impregnated columns were used to identify CLA isomers based on different retention times of a standard. The methodology is described in detail by Dannenberger, Nuernberg and Nuernberg (2009).

Microsomal extraction and stearoyl-CoA desaturase (SCD) assay

Samples of *longissimus* muscle and subcutaneous adipose tissue (SAT) were homogenized in a sucrose buffer and after two centrifugation steps the microsomes were resuspended in an ice-cold phosphate buffer and were stored by -80°C.

To determine the SCD activity in tissues the conversion rate of labeled palmitoyl-CoA to labeled palmitoleic acid was measured. This method was done as described by Doran, Moule, Teye, Whittington, Hallett and Wood (2006). The radioactivity was counted using a LSA Tri-Carb liquid scintillation counter from Perkin Elmer.

German corned beef sausages (GCB) and Tea sausage spreads (TSS)
GCB and TSS were produced by Greifenfleisch GmbH (Greifswald, Germany). GCB contains 58 % beef from bulls of this experiment (lean meat from joint and bug), 5 % beef rind, and drinking water, gelatine, pickling salt, spices, yeast extracts, celeriac, and corn, soy and plant protein. TSS contain 94 % beef and pork meat, pickling salt, spices, lactose, sugar, dextrose, rum, antioxidant E 300 and E 301, flavor enhancer and beech wood fume. From each carcass single sausages were produced and in total 29 GCB and 29 TSS were analyzed.

The data were analyzed by the least-squares method using the general linear model procedures (**GLM**) of SAS® *(24)* with the fixed factor feeding.

III. RESULTS AND DISCUSSION

Longissimus muscle and subcutaneous adipose tissue(SAT)
The experimental diet induced a significantly lower amount of all fatty acids shown in Table 1 except C18:1*trans*-11 (TVA) and the *n*-3 fatty acids in *longissimus* muscle. TVA was not altered by the diets and the *n*-3 fatty acids, C18:3*n*-3 and LC PUFA, are significantly higher in the experimental compared to the control group.

Table 1: Fatty acid composition in mg/100 g of *longissimus* muscle and subcutaneous adipose tissue of German Holstein bulls

	Longissimus muscle		Subcutaneous adipose tissue	
	Control group LSM±SEM	Experimental group LSM±SEM	Control group LSM±SEM	Experimental group LSM±SEM
C16:0	627.2±52.0^{a}	448.1±53.8^{b}	18742.2±796.9^{A}	16532.8±824.8^{B}
C16:1*cis*-9	89.2±8.1^{a}	57.9±8.3^{b}	4561.0±259.1	4196.6±268.1
C18:0	342.6±25.6^{A}	277.4±26.5^{B}	8102.3±537.4	7149.8±556.3
C18:1*cis*-9	892.8±76.3^{a}	614.9±79.0^{b}	25194.4±955.5^{a}	21628.6±989.0^{b}
C18:1*trans*-11	13.6±1.2	13.9±1.2	543.8±37.3	565.2±38.6
C18:2*n*-6 (LA)	112.9±3.3^{a}	95.2±3.4^{b}	1176.1±49.4^{a}	843.7±51.1^{b}
C18:3*n*-3 (ALA)	13.0±1.1^{a}	33.4±1.1^{b}	281.4±27.8^{a}	418.4±28.8^{b}
C20:4*n*-6	29.9±1.0^{a}	26.4±1.0^{b}	37.5±2.7^{a}	29.0±2.7^{b}
C20:5*n*-3 (EPA)	3.9±0.3^{a}	8.8±0.3^{b}	3.0±1.5^{a}	10.5±1.5^{b}
C22:4*n*-6	4.6±0.1^{a}	2.5±0.2^{b}	22.0±2.4^{a}	9.7±2.5^{b}
C22:5*n*-3	8.4±0.3^{a}	12.0±0.3^{b}	29.5±3.7	31.6±3.8
C22:6*n*-3 (DHA)	1.0±0.1^{a}	1.4±0.1^{b}	3.9±2.4	3.2±2.5
Σ SFA	1078.7±84.8^{a}	805.5±87.7^{b}	31260.2±1456.3^{A}	27576.9±1507.4^{B}
Σ MUFA	1083.8±92.1^{a}	752.0±95.3^{b}	33238.5±1198.4^{a}	28842.7±1240.4^{b}
Σ *n*-3 FA	27.5±1.4^{a}	56.5±1.4^{b}	317.8±31.6^{a}	463.7±32.7^{b}
Σ *n*-6 FA	157.6±4.3^{a}	131.5±4.4^{b}	1316.1±50.7^{a}	932.9±52.5^{b}
Ratio *n*-6/*n*-3 FA	5.8±0.1^{a}	2.3±0.1^{b}	4.6±0.3^{a}	2.1±0.3^{b}

a,b means P≤0.05; A,B means P≤0.1

These results are in line with several other studies in which feeding grass silage and concentrate supplemented with linseed, rapeseed and/or algae or natural grazing have lead to an accumulation of beneficial *n*-3 fatty acids in ruminant tissues (Wood *et al.* 2008). The higher amount of *n*-3 fatty acids in the experimental group resulted in a lower *n*-6/*n*-3 ratio (2.3±0.1) and is in accordance with the recommendation of the German Nutrition Society (*n*-6/*n*-3 FA ratio of ≤ 5:1) (DGE 2008). The SFA and MUFA content of the *longissimus* muscle in experimental bulls is significantly lower compared to the control group. This result and similar amount of CLA*cis*-9,*trans*-11 could be attributed to the inhibition of the SCD, the key enzyme in formation of MUFA starting from SFA (Perfield *et al.* 2007) and formation of CLA*cis*-9,*trans*-11 starting from TVA (Smith, Lunt, Chung, Choi, Tume & Zembayashi 2006). Another important CLA isomer is CLA*trans*-10,*cis*-12. This isomer has shown anti-obesity effects in obese mice (Lee, Paek, Lee, Park & Lee 2007). The significantly lower amount in muscle of the experimental group is in line with results received in feeding studies with goat muscles (Bernard, Shingfield, Rouel, Ferlay & Chilliard 2009) and milk of British Holstein Friesian cows (Shingfield *et al.* 2005).
The fatty acid composition in SAT is also shown in Table 1. As in muscle the SFA and MUFA content is in the experimental group significantly lower compared to the control group. The SCD inhibition could be the reason for this difference (Herdmann *et al.* 2010). The higher amount of ALA (P≤0.05) in the experimental group due to the grass silage and the concentrate supplemented with linseed oil and rapeseed cake corresponds to the results from Fincham *et al.* (2009). They observed an increase of ALA in ruminal fluid and SAT and a decrease of LA in pasture-finished cattle. In our study the amount of LA is significantly lower in experimental group but the higher amount in the control group is also attributed to the higher amount of LA in the diet.

German corned beef sausages (GCB) and Tea sausage spread(TSS)

The fresh meat intake in Germany is noted with 42 g/day for men and 23 g/day for women. However, the consumption of processed meat is about 61 g/day for men and 30 g/day for women (Federal Ministry of Food, Agriculture and Consumer Protection 2008). Therefore, it is of interest to investigate the transfer of beneficial fatty acids during the processing. In both sausages the amount of the sum of *n*-3 fatty acids, EPA and DPA are significantly higher in the experimental group whereas in the GCB additionally the DHA is significantly increased in the experimental group. Less information in the literature are available about the concentration of *n*-3 fatty acids in fresh muscle and corresponding products. The PUFA concentration in grilled pork slices was significantly increased due to the water loss compared to the fresh meat (Nuernberg, Kuechenmeister, Nuernberg, Hartung, Dannenberger & Ender 2006) and the cooking of meat from grazing heifers did not result in detrimental changes of the fatty acid composition (Sarriés, Murray, Moloney, Troy & Beriain 2009). The *n*-6/*n*-3 ratio of GCB made from meat of the experimental group corresponds with 4.0±0.4 to the recommendation (≤5:1) of the DGE (2008). Considering the *n*-6/*n*-3 ratio of TSS it is with 6.4±0.3 higher than the recommendation but the sausage of the experimental group also contains a significantly higher amount of ALA and DPA and this despite the proportion of conventional produced porcine meat.

Table 2: Fatty acid composition in mg/100 g of German corned beef sausage and Tea sausage spread

	German corned beef sausage		Tea sausage spread	
	Control group LSM±SEM	Experimental group LSM±SEM	Control group LSM±SEM	Experimental group LSM±SEM
C16:0	491.4±28.1	435.4±29.1	5367.8±139.2	5283.2±144.1
C16:1*cis*-9	81.9±5.2^{A}	67.2±5.4^{B}	789.5±23.6^{a}	716.7±24.5^{b}
C18:0	297.6±16.3	290.4±16.8	3355.4±107.0	3486.5±110.7
C18:1*cis*-9	840.2±49.6	738.7±51.4	8310.8±263.0	8350.0±272.2
C18:1*trans*-11	15.7±1.4	15.4±1.5	108.3±7.3	97.5±7.6
C18:2*n*-6 (LA)	167.9±14.8	150.8±15.3	1635.9±94.3	1683.0±97.6
C18:3*n*-3 (ALA)	21.4±2.8^{A}	29.4±2.9^{B}	176.8±13.5^{a}	230.3±14.0^{b}
C20:4*n*-6	28.2±1.7	28.6±1.8	45.5±2.4	46.0±2.4
C20:5*n*-3 (EPA)	4.0±0.7^{a}	7.5±0.7^{b}	5.3±0.3^{A}	6.22±0.3^{B}
C22:4*n*-6	4.9±0.3^{a}	3.5±0.3^{b}	17.2±0.9	16.0±0.9
C22:5*n*-3	8.9±0.7^{a}	12.2±0.8^{b}	17.8±0.8^{a}	20.4±0.8^{b}
C22:6*n*-3 (DHA)	1.1±0.1^{a}	1.5±0.1^{b}	5.3±0.5	5.4±0.5
Σ SFA	867.5±47.3	799.3±49.0	9523.5±241.5	9552.3±250.0
Σ MUFA	1024.1±59.9	898.0±62.0	10172.9±303.9	10092.2±314.5
Σ *n*-3 FA	38.2±4.1^{a}	53.3±4.3^{b}	228.2±15.3^{a}	289.2±15.8^{b}
Σ *n*-6 FA	213.7±15.7	194.1±16.2	1782.3±100.7	1832.8±104.3
Ratio *n*-6/*n*-3 FA	5.9±0.4^{a}	4.0±0.4^{b}	8.0±0.3^{a}	6.4±0.3^{b}

While the CLA*trans*-10,*cis*-12 (Figure 1) is significantly lower in muscle fat, SAT and in the two sausages of the experimental animals, the amount of the main isomer CLA*cis*-9,*trans*-11 is not different in all tissues between both feeding groups. It seems there was no loss of fatty acids (CLA isomers and the beneficial *n*-3 fatty acids) during processing of fresh meat to two beef products, GCB and tea sausage spread.

SCD enzyme activity in longissimus muscle and subcutaneous adipose tissue(SAT)

The SCD enzyme activity in both tissues is shown in Figure 2. The specific enzyme activity in MLD for experimental group is with 0.4±0.1 nmol palmitoleic acid/mg protein/hour significantly lower than in control group with 0.8±0.1 nmol palmitoleic acid/mg protein/hour. The SCD activity in SAT is with 168.6±18.6 nmol palmitoleic acid/mg protein/hour for control and 101.6±21.7 nmol palmitoleic acid/mg protein/hour for experimental group much higher compared to the activity in muscle.

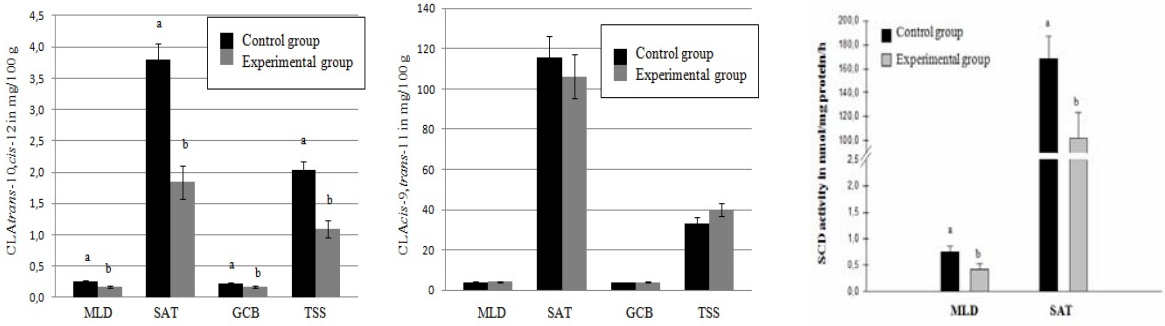

Figure 1: CLA*trans*-10,*cis*-12 and CLA*cis*-9,*trans*-11 in mg/100 g in *longissimus* muscle (MLD), subcutaneous adipose tissue (SAT), German corned beef sausages (GCB) and Tea sausage spread (TSS)

(a,b- sign. differences between groups within muscle and within subcut. fat at P≤0.05)

Figure 2: Specific SCD enzyme activity in *longissimus* muscle and subcutaneous adipose tissue of German Holstein bulls

These results are in line with the significantly lower amount of C18:1*cis*-9 in both tissues of the experimental group, which is synthesized by SCD (Ntambi 1999). Waters *et al.* (2009) and Howell *et al.* (2009) found out that rather *n*-3 LC PUFAs like EPA and DHA inhibit the SCD *via* SREBP in different cell lines. In the present study both fatty acids are increased in the muscle and EPA only in SAT of experimental animals and confirm the results mentioned above. That the SCD activity in SAT is clearly higher than in MLD is also demonstrated by Archibeque, Lunt, Gilbert, Tume and Smith (2005).

IV. CONCLUSION

Feeding an ALA enriched diet to German Holstein bulls resulted in an enhancing of this beneficial fatty acid and the sum of *n*-3 LC PUFA in MLD, SAT and in products. The specific SCD activity is significantly reduced in MLD and SAT due to the ALA enriched diet resulting in a reduced amount of C18:1*cis*-9 in MLD and SAT of the experimental group. The concentration of the main isomer CLA*cis*-9,*trans*-12 is not different between both groups. Most importantly, the processing did not lead to a loss of beneficial PUFA in GCB. The GCB is rich in *n*-3 PUFAs and the ratio *n*-6/*n*-3 PUFA corresponds to the recommendation of the DGE. Despite the amount of porcine meat the concentration of ALA in TSS is high and could contribute to an adequate supply of *n*-3 fatty acids in the human nutrition.

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